UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 27 1987 005794

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Review of the Mouse Oncogenicity Study on 2,4-Dichlorophenoxyacetic acid

From: Marcia van Gemert, Ph.D. M. vanGemert 3/23/87
Head, Section III
Toxicology Branch, HED (TS-769C)

To: Lin Vlier
Registration Division (TS-767C)

Thru: Theodore M. Farber, Ph.D. Theodore M. Farber 3/27/87
Chief, Toxicology Branch, HED

Compound: 2,4-Dichlorophenoxyacetic acid

Tox Chem No: 315

Registrant: Industry Task Force on 2,4-D Research Data

Accession No: 400618-01

Action Requested: The Toxicology Branch has been requested to review the recently submitted mouse oncogenicity study on 2,4-Dichlorophenoxyacetic acid (2,4-D). This review, along with the chronic/oncogenicity study in rats will be presented before the Peer Review Committee April 21, 1987. The conclusions reached from the review of this study are presented below.

Conclusions: 2,4-D at doses of 0, 1, 15 and 45 mg/kg were fed to $B_6C_3F_1$ mice for 104 weeks with a 52 week interim sacrifice. Effects were seen in absolute and relative kidney and adrenal weights at 15 and 45 mg/kg. Histopathology revealed an increase in the mid and high dose groups in cytoplasmic homogeneity of the renal tubular epithelium due to a reduction of cytoplasmic vacuoles.
NOEL = 1 mg/kg
LEL = 15 mg/kg based on treatment-related kidney and adrenal effects
Classification: core minimum; batch number and dietary stability and homogeneity data were not provided.

Reviewed by: Marcia van Gemert, Ph.D. M. van Gemert 3/20/87
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D. Theodore M. Farber 3/27/87
Chief, Tox. Branch (TS-769C)

005794

DATA EVALUATION REPORT

STUDY TYPE: Mouse Oncogenicity study TOX. CHEM. NO.: 315

ACCESSION NUMBER: 400618-01 MRID NO.: 40061801

TEST MATERIAL: 2,4-dichlorophenoxyacetic acid

SYNONYMS: 2,4-D

STUDY NUMBER(S): 2184-101

SPONSOR: Industry Task Force for 2,4-D Research Data

TESTING FACILITY: Hazleton Laboratories America Inc.
9200 Leesburg Turnpike, Vienna Va. 22180

TITLE OF REPORT: Oncogenicity Study in Mice with 2,4-dichlorophenoxyacetic acid (2,4-D)

AUTHOR(S): D.G. Serota

REPORT ISSUED: Not dated, but compliance statement was signed and dated by study director 1/15/87

CONCLUSIONS: 2,4-D at doses of 0, 1, 15, and 45 mg/kg were fed to $B_6C_3F_1$ mice for 104 weeks with a 52 week interim sacrifice. Effects were seen in absolute and relative kidney and adrenal weights at 15 and 45 mg/kg. Histopathology revealed an increase in the mid and high dose groups in cytoplasmic homogeneity of the renal tubular epithelium due to a reduction of cytoplasmic vacuoles.
NOEL = 1 mg/kg
LEL = 15 mg/kg based on treatment-related kidney and adrenal effects.

Classification: core-Minimum, batch number and dietary stability and homogeneity data were not provided.

Special Review Criteria (40 CFR 154.7)

005794

A. MATERIALS:

1. Test compound: 2,4-dichlorophenoxyacetic acid (2,4-D)
Description: beige powder
Batch # not given, Purity 97.5%, contaminants: listed in CBI appendix

2. Test animals: Species: mice, Strain: B_6 C_3F_1Crl Br
Age: 7 weeks at initiation of study
Weight: males: 16.4-24.2 gms, females: 14.8-20.9 gms.
Source: Charles River Breeding Laboratories Inc. Kingston, N.Y.

B. STUDY DESIGN:

1. Animal assignment

240 animals/sex were assigned randomly to the following test groups:

Test Group	Dose in diet mg/kg	Main Study 24 months male	female	Interim Sac. 52 weeks male	female
1 Cont.	0	50	50	10	10
2 Low (LDT)	1	50	50	10	10
3 Mid (MDT)	15	50	50	10	10
4 High(HDT)	45	50	50	10	10

2. Diet preparation

Diet was prepared weekly. The concentration of test material in the diet was adjusted weekly through the first 15 weeks and every 4th week thereafter. Reserve samples were taken from the initial batch received and from each mixed batch of test diet and sent to the sponsor. Additional reserve samples were taken from each mixed batch of diet and retained under refrigeration.

Results - Stability data were not provided. A statement in the study text was made that "the sponsor provided information that 2,4-D was stable in the diet for at least one month. Homogeneity of 2,4-D in the diet was established in previous studies conducted at Hazleton Biotechnologies Corp."

Routine analysis conducted during the test on 2,4-D mouse study gave the following results on table I.

005794

TABLE I

group	sex	Percent of target range low	Percent of target range high	percent of target mean ± S.D.
2	male	73.99	136.1	96.48 ± 14.824
2	female	77.92	121.2	97.57 ± 10.677
3	male	85.17	111.5	97.56 ± 6.490
3	female	80.91	110.0	95.43 ± 8.234
4	male	80.24	103.4	95.61 ± 4.966
4	female	78.79	101.9	94.26 ± 5.341

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - The procedures utilized in analyzing the numerical data are on appended pages 3-7.

5. Quality assurance statement was signed by Frederick G Snyder and dated 1/7/87.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. Detailed physical examinations for unusual appearance and behavior, and palpation of tissue masses and abdominal distension were performed weekly for weeks 1-14 and biweekly thereafter.

Mortality (survival) There were no statistically significant differences between treated and controls concerning mortality. Appended pages 8 and 9 graphically represent survival data. The numbers of animals dying on test are tabulated in table II.

TABLE II
Animals which died or were sacrificed in extremis prior to week 104

Group	males	females[c]
1	10	12
2	6	8[b]
3	10	17
4	11[a]	15

[a] One found out of cage at week 30 and was removed from study.
[b] One was found dead during week 105 prior to scheduled sacrifice
[c] 3 animals (2 group 2, one group 3) were missexed and discovered at week 2 and removed from the study

005794

Toxicity: No treatment-related effects were seen.

2. Body weight

Animals were weighed at initiation of study and weekly for weeks 1-14, and biweekly thereafter.

Results: There were no treatment-related effects on body weight or body weight gains. Appended pages 10 and 11 graphically represent body weight data.

3. Food consumption and compound intake

Consumption was determined weekly for weeks 1-14 and biweekly thereafter. Data on food efficiency and compound intake were not given.

Results:
There did not appear to be any treatment-related changes associated with food consumption. There was a significant increase in food consumption in males at weeks 1-52 and 1-104 weeks in groups 2 and 4. Data are presented in Table IV below. Females in group 2 at 1-104 weeks showed an increase in food consumption. Females for weeks 1-52 showed a positive trend in food consumption.

TABLE IV

weeks	males				females			
	1	2	3	4	1	2	3	4
1-52	1361.8	1419.1*	1368.0	1418.9*	1485.0	1536.3	1532.2	1523.9
SD	91.97	87.51	84.19	104.6	91.79	119.66	109.61	109.74
N	56	59	54	50	53	47	50	56
1-104	2313.8	2439.0*	2349.6	2437.6*	2542.6	2668.2*	2568.3	2607.6
SD	137.41	139.7	134.81	170.54	142.91	146.68	178.84	178.09
N	31	35	35	32	30	29	27	33

* Significantly different from controls $p < 0.05$.

4. Ophthalmological examinations were not performed.

005794

5. Blood was collected for hematology from the last surviving 10 mice/sex/group following 52 weeks of treatment and from the first 10/sex/group following 104 weeks of treatment. Clinical Chemistry analyses were not performed. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood Clotting Measurements	X	Cell morphology

* Required for subchronic and chronic studies

Results: No compound-related changes in hematological parameters were seen.

6. Urinalysis was not performed.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue		.Aorta*	XX	.Brain*† at least 3 levels
X	.Salivary glands*	XX	.Heart*	X	Periph. nerve*#
X	.Esophagus*	X	.Bone marrow*	X	Spinal cord (3 levels)*#
X	.Stomach*	X	.Lymph nodes*[6]	XX	.Pituitary*[5]
X	.Duodenum*	X	.Spleen*	X	Eyes (optic n.)*#[7]
X	.Jejunum*	X	.Thymus* (when presnt)		Glandular
X	.Ileum*		Urogenital	XX	.Adrenals*[5]
X	.Cecum*	XX	.Kidneys*†[3]		Lacrimal gland#
X	.Colon*	X	.Urinary bladder*	X	Mammary gland*#
	.Rectum*	XX	.Testes*†[2]		.Parathyroids*††
XX	.Liver*†[1]		Epididymides	XX	.Thyroids*††[4]
	Gall bladder*#	X	Prostate		Other
X	.Pancreas*		Seminal vesicle	X	Bone*# Sternum w marrow
	Respiratory	XX	Ovaries*†[5]	X	Skeletal muscle*#
X	.Trachea*	X	.Uterus*	X	Skin*#
X	.Lung*			X	All gross lesions
				X	3 coronal sections[8]

Footnotes are on the next page

005794

* Required for subchronic and chronic studies
° Required for chronic inhalation
In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

1 weighed with gall bladder
2 weighed with epididymides
3 left and right (weighed separately, combined weight was calculated
4 weighed with parathyroids post fixation, and sectioned together
5 weighed post fixation
6 all grossly enlarged or otherwise abnormal lymph nodes, or nodes draining known or suspected tumor sites.
7 with contiguous harderian gland
8 coronal sections through head to include nasal cavity, paranasal sinuses, oral cavity, nasopharynx and middle ear

Gross examination included examination of :
external surface, all orifices, cranial cavity, carcass, nasal cavity and paranasal sinuses (not opened at necropsy but opened and examined post fixation, except for 10 animals/sex/group selected for histopathology exam of the spinal cord and coronal head sections), cervical tissues and organs, thoracic, abdominal and pelvic cavities and their viscera, cut surface of brain and external and cut surfaces of the spinal cord (examined post fixation during tissue trimming) according to the study text. All preserved tissues (except spinal cord, skeletal muscle and coronal sections of the head) from all mice were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically. The spinal cord (cervical and thoracic) and coronal sections through the head were examined microscopically from the last 10 animals/sex/group surviving to study termination.

005794

a. Organ weight: Both kidneys and adrenals appeared to be affected by treatment.

Kidneys: absolute kidney weights, both left and right as well as combined, were significantly increased in group 4 females, and all revealed a significant positive trend. There were significantly increased kidney/brain weight ratios in group 4 females for left right and combined kidneys. At 104 weeks all absolute and relative kidney weight parameters were significantly increased weight in group 4 females with most significant positive trends seen, except kidney/brain weights for combined kidneys. Kidney/body weight ratios in group 3 females for left, right and combined kidneys were significantly elevated. Group 4 males also showed an increase in kidney/body weight ratios, possibly due to the drop in body weight seen at high dose. Data for the 52 week sacrifice are in table V, and data for the terminal sacrifice are in table VI.

Adrenals: Absolute and all relative male adrenal weights were decreased at all doses tested at week 54, with significant trends seen for all parameters. At week 104, all parameters in the mid and high dose groups were increased (rather than decreased at 54 weeks) with significant positive trends seen in all parameters for males. Data are given in table V and VI.

TABLE V

005794

ORGAN WEIGHTS 52 WEEK SACRIFICE

left kidney male	#	Abs Wts Mean	SD	Organ-to body wts. mean	S.D.	Organ-to Brain wts. Mean	S.D.
1	10	0.28	0.03	0.975	0.068	0.622	0.067
2	10	0.29	0.03	1.006	0.073	0.632	0.056
3	10	0.28	0.03	0.899	0.061	0.627	0.062
4	10	0.28	0.02	0.873*a	0.091	0.617	0.041
left kidney female							
1	10	0.20	0.02	0.763	0.065	0.436	0.033
2	10	0.20	0.02	0.742	0.092	0.420	0.036
3	10	0.20	0.01	0.749	0.076	0.435	0.029
4	10	0.22*a	0.02	0.825	0.116	0.471*	0.032
right kidney male							
1	10	0.29	0.02	1.014	0.048	0.647	0.064
2	10	0.31	0.02	1.074	0.093	0.673	0.039
3	10	0.30	0.03	0.952*	0.051	0.665	0.065
4	10	0.30	0.02	0.923a	0.118	0.651	0.049
right kidney female							
1	10	0.21	0.02	0.794	0.063	0.455	0.045
2	10	0.22	0.02	0.801	0.079	0.454	0.031
3	10	0.22	0.01	0.802	0.083	0.466	0.027
4	10	0.24*a	0.02	0.893*	0.099	0.512*a	0.040

Combined kidneys males	X	Abs wts mean	S.D.	Organ-to body wts mean	S.D.	Organ-to Brain wts Mean	S.D.
1	10	0.58	0.05	1.991	0.111	1.270	0.125
2	10	0.60	0.05	2.085	0.169	1.307	0.084
3	10	0.58	0.05	1.845*	0.100	1.288	0.123
4	10	0.58	0.04	1.793	0.206	1.266	0.086
Combined kidneys females							
1	10	0.42	0.04	1.558	0.119	0.892	0.079
2	10	0.42	0.04	1.552	0.169	0.879	0.063
3	10	0.42	0.02	1.546	0.153	0.899	0.055
4	10	0.46*a	0.03	1.716	0.207	0.982*	0.061
Adrenal males							
1	10	0.007	0.002	0.0258	0.0068	0.0163	0.0039
2	10	0.005*	0.001	0.0189*	0.0047	0.0117*	0.0026
3	10	0.005*	0.001	0.0164*	0.0023	0.0114*	0.0016
4	10	0.006*a	0.001	0.0177*a	0.0047	0.0125*a	0.0030
Adrenal females							
1	10	0.008	0.001	0.0306	0.0041	0.0175	0.0023
2	10	0.008	0.001	0.0290	0.0036	0.0164	0.0009
3	10	0.008	0.001	0.0299	0.0060	0.0173	0.0027
4	10	0.008	0.003	0.0304	0.0137	0.0170	0.0065

* Significantly different from controls ($p \leq 0.05$)
a Significant trend ($p \leq 0.05$)

005794

TABLE VI

Organ Weights for the Terminal Sacrifice

Left Kidney males	X	Abs Wts Mean	S.D.	Organ-to Body Wts. Mean	S.D.	Organ-to Brain Wts Mean	S.D.
1	40	0.32	0.04	1.049	0.104	0.707	0.068
2	44	0.32	0.03	1.093	0.115	0.712	0.058
3	40	0.32	0.03	1.095	0.098	0.712	0.064
4	38	0.33	0.03	1.135*[a]	0.112	0.729	0.065
left kidney females							
1	38	0.22	0.02	0.819	0.090	0.469	0.044
2	38	0.23	0.02	0.857	0.091	0.486	0.051
3	31	0.23	0.02	0.880*	0.079	0.487	0.055
4	34	0.27*[a]	0.02	1.003*[a]	0.092	0.559*[a]	0.049
Right kidney males							
1	40	0.34	0.04	1.106	0.102	0.745	0.069
2	44	0.34	0.03	1.142	0.112	0.744	0.060
3	40	0.34	0.03	1.139	0.106	0.740	0.068
4	38	0.35[a]	0.03	1.185*[a]	0.106	0.762[a]	0.069
Right kidney females							
1	38	0.24	0.02	0.883	0.091	0.505	0.043
2	38	0.24	0.03	0.903	0.082	0.510	0.048
3	31	0.25	0.03	0.952*	0.122	0.527	0.079
4	33	0.28*	0.03	1.042*[a]	0.101	0.581*	0.048
Combined kidneys males							
1	40	0.67	0.07	2.153	0.180	1.450	0.122
2	44	0.66	0.06	2.236	0.216	1.457	0.109
3	40	0.66	0.05	2.234	0.194	1.453	0.123
4	38	0.68	0.06	2.322*[a]	0.208	1.492	0.129
Combined kidneys females							
1	38	0.46	0.04	1.701	0.178	0.973	0.084
2	38	0.47	0.04	1.762	0.167	0.998	0.094
3	31	0.48	0.05	1.832*	0.191	1.014	0.128
4	33	0.55*[a]	0.05	2.045*[a]	0.182	1.141*	0.092
Adrenals male							
1	40	0.005	0.002	0.0157	0.005	0.0107	0.0040
2	43	0.005	0.002	0.0182	0.0063	0.0118	0.0042
3	40	0.006*	0.002	0.0214*	0.0078	0.0138*	0.0047
4	37	0.006*[a]	0.002	0.0213*[a]	0.006	0.0135*[a]	0.0035
Adrenals female							
1	37	0.008	0.002	0.0283	0.0083	0.0164	0.0052
2	38	0.007	0.002	0.0280	0.006	0.0158	0.0037
3	31	0.007	0.002	0.0277	0.0075	0.0153	0.0043
4	35	0.008	0.002	0.0288	0.0073	0.0162	0.0044

* Significantly different from controls $p \leq 0.05$

[a] Significant trend $p \leq 0.05$

005794

b. Gross pathology: No treatment-related effects were noted in the unscheculed deaths, interim sacrifice and terminal sacrifice.

c. Microscopic pathology

1) Non-neoplastic:

Kidney: There was a compound related increase in histomorphologic alteration in the renal tubule epithelium of males. This was characterized as a cytoplasmic homogeneity, and was due to the reduction of cytoplasmic vacuoles that are normally present in the renal tubular epithelium. These effects are presented in Table VII. Other lesions seen were not treatment-related and were the result of normal spontaneous disease processes associated with the $B_6 C_3F_1$ mouse.

TABLE VII

Incidence of Cytoplasmic Homogeneity- Increased Tubular Epithelium

Group/sex	1m	2m	3m	4m	1f	2f	3f	4f
Unscheduled deaths								
N	10	6	10	11	0	0	0	0
Increased Tubular Epi.	3	5*	10***	10***	0	0	0	0
52-week sacrifice								
N	10	10	10	10	10	10	10	10
Increased Tubular Epi.	0	1	4*	10***	0	0	0	0
Terminal sacrifice								
N	40	44	40	38	38	38	31	35
Increased Tubular Epi.	8	9	34***	38***	0	0	0	0
Combined deaths								
N	60	60	60	59	60	58	59	60
Increased Tubular Epi.	11	15	48***	58***	0	0	0	0

* Significantly different from Controls $p < 0.05$
** Significantly different from Controls $p < 0.01$
*** Significantly different from Controls $p < 0.001$
Fishers Exact Test used by reviewer

Adrenal: No histopathological findings were noted in the adrenal at the interim sacrifice or terminal sacrifice, or in unscheduled deaths.

2) Neoplastic: No treatment-related increased incidence in tumors was seen at the 52 week sacrifice, in unscheduled deaths, at terminal sacrifice, or in combined scheduled and unscheduled deaths.

Discussion:

005794

There were no significant treatment-related findings in toxicity, mortality, body weights, food consumption, hematology and gross pathology. Kidney and adrenal weights were effected by 2,4-D treatment. At 52 weeks there was an increase in high dose females in combined absolute kidney and kidney/brain weight ratios with a significant trend seen in absolute weights. All male adrenal weight parameters were elevated at all doses at 52 weeks with significant trends seen in all parameters. At 104 weeks, female and male kidney weights were elevated at the high dose with significant trends in female absolute kidney and organ/brain weight ratios and organ/body weight in males. Female organ/body weight ratios were elevated at the mid dose also. All adrenal organ weight parameters were elevated for males in both the mid and high dose groups at 104 weeks with significant trends seen in all parameters. Histopathology revealed a treatment-related histomorphologic alteration of the renal tubular epithelium of males seen mainly in the mid and high dose groups. The unscheduled deaths showed a marginal increase in tubular epithelium alteration at the low dose. No other treatment-related effects were evident and no increase in treatment-related tumors was seen.
The adrenal weight data indicated significant increases in weight at all doses at the 52 week sacrifice. However, at terminal sacrifice the low dose animals appeared similar to controls. No histopathologic signs were evident either at 52 weeks or at terminal sacrifice, so the henomen may have been stress induced. Since the low dose showed no effects at terminal sacrifice, the 1 mg/kg was considered the NOEL.

NOEL = 1 mg/kg
LEL = 15 mg/kg based on treatment-related kidney and adrenal effects

appended pg 1

"Contains or relates to proprietary information of the Industry Task Force on 2,4-D Research Data"

initiation of treatment. Extra animals not assigned to study were euthanatized and appropriately discarded.

Compound Preparation and Administration

Test material for each dietary level was weighed on an Arbor balance and premixed with approximately 200 grams of basal diet. The premix was blended in a Waring blender for approximately 2 minutes or until a homogeneous mixture was achieved. Each dietary level of premix was added to appropriate amounts of basal diet and mixed in a Patterson-Kelly Twin Shell mixer fitted with an intensifier bar at a rate of one minute/kg of diet. For purposes of dosage calculations, the purity was adjusted to 100%. Fresh diets were prepared and presented weekly throughout the study. The concentration of 2,4-D in the diet was adjusted weekly through the first 15 weeks and every fourth week thereafter. Reserve samples of the test material (10 g) received on March 15, 1982, were taken at initiation and at termination of the study and shipped to the sponsor. In addition, reserve samples from each mixed batch of test diet and the control feed (200 g each) were taken and retained under refrigeration.

Diets containing appropriate amounts of 2,4-D as well as control diets were administered to the animals *ad libitum*. Dietary administration of 2,4-D was performed because potential human exposure is by the oral route.

Sampling and Analysis of Diets and Water

A 200 gram reserve sample of the diet from each mixed batch was retained and frozen. The certified diet was analyzed by the manufacturer for concentrations of heavy metals, aflatoxins, organophosphates, chlorinated hydrocarbons, and specified nutrients. Water analyses were provided by Fairfax County Water Authority. The results of both are on file with the Department of Laboratory Animal Medicine at Hazleton Laboratories America, Inc.

The sponsor provided information that 2,4-D was stable in the diet for at least one month. Homogeneity of 2,4-D in the diet was established in previous studies conducted at Hazleton Biotechnologies Corporation (Nos.

2184-100 and 2184-102). Concentration of 2,4-D in the diet was analyzed by the Analytical Chemistry Department of Hazleton Biotechnologies Corporation for Weeks 1, 2, 3, 4, 17, 30, 43, 56, 69, 82, and 95. The analytical method used for determination of 2,4-D in the diet was jointly developed by Dow Chemical Company and Hazleton Biotechnologies Corporation, Inc. and is presented in Appendix 8.

Observations and Records

All animals were observed at least twice daily for mortality and moribundity with rare exception. Detailed physical examinations for unusual appearance and behavior, and palpation for tissue masses and abdominal distension were performed weekly for Weeks 1 through 14 and biweekly thereafter. Individual body weights were recorded at initiation of study, and body weight and food consumption measurements were recorded weekly for Weeks 1-14 and biweekly thereafter.

Clinical Pathology

Clinical hematology samples were collected from the last surviving 10 mice/sex/group following 52 weeks of treatment and from the first surviving 10 mice/sex/group following 104 weeks of treatment. Blood was collected by orbital sinus bleeding after an overnight fast from food, using ethylenediaminetetraacetic acid (EDTA) as the anticoagulant.

The following parameters were determined:

Hematology

Erythrocyte count (RBC)
Reticulocyte count (RETIC)[a]
Hemoglobin (HGB)
Hematocrit (HCT)
Platelet count (PLATELET)
Total leukocyte count (WBC)
Differential leukocyte count (BLAST, META, BAND, SEG, LYMPH, MONO, EOSIN, BASO)
Cell morphology

[a] In addition, an absolute reticulocyte count (A RETIC) was calculated.

three coronal sections through the head to include nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx, and middle ear

Histopathology

All of the preserved tissues (except the spinal cord, skeletal muscle, and coronal sections of the head) from all of the mice were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically. The spinal cord (cervical and thoracic) and coronal sections through the head were examined microscopically from the last 10 animals/sex/group surviving to study termination. The lumbar spinal cord and skeletal muscle were not examined.

Statistical Analyses

Cumulative survival data through Week 104 were analyzed using the National Cancer Institute Package. Trend analysis of survival was evaluated at the 5.0% one-tailed probability level.

Growth rates (rates of body weight gain) were compiled using body weight values from Weeks 0, 3, 9, 18, 30, and 46 for males and Weeks 0, 2, 6, 14, 26, and 46 for females (Rao, 1958).

Absolute body weights at Weeks 52 and 104, body weight changes between Weeks 0 and 52 and Weeks 0 and 104, total food consumption through Weeks 52 and 104, clinical hematology data (except cell morphology), and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups. Statistical analyses were performed as diagrammed in Figures 1A (groupwise comparison) and 1B (trend) for all body weight and food consumption parameters; and in Figure 1C (for both groupwise comparison and trend analyses) for the clinical hematology and organ weight data.

The initial test used was Levene's test for homogeneity of variances. Homogeneous data were further analyzed using a one-way classification analysis of variance (ANOVA). When the variances were heterogeneous, a series of transformations was performed on the data, until appended pg 4

005794

Figure 1A

LEVENE'S TEST OF HOMOGENEITY OF VARIANCES

Homogeneous

Heterogeneous

<------Homogeneous------------ LOG_{10} TRANSFORMATION

Heterogeneous

<------Homogeneous------------ SQUARE TRANSFORMATION

Heterogeneous

<------Homogeneous--------- SQUARE ROOT TRANSFORMATION

Heterogeneous

<------Homogeneous---------- RECIPROCAL TRANSFORMATION

Heterogeneous

<------Homogeneous------ ANGULAR (ARCSINE) TRANSFORMATION

Heterogeneous

<------Homogeneous------------ RANK TRANSFORMATION

<-------------Heterogeneous-------------

ANOVA

Not Significant

STOP

Significant

DUNNETT'S CONTROL VS TREATMENT COMPARISONS (FOR EQUAL VARIANCES, FOR UNEQUAL VARIANCES, IF HETEROGENEOUS)

"Contains or relates to proprietary information of the Industry Task Force on 2,4-D Research Data"

005794

005794

Figure 1B

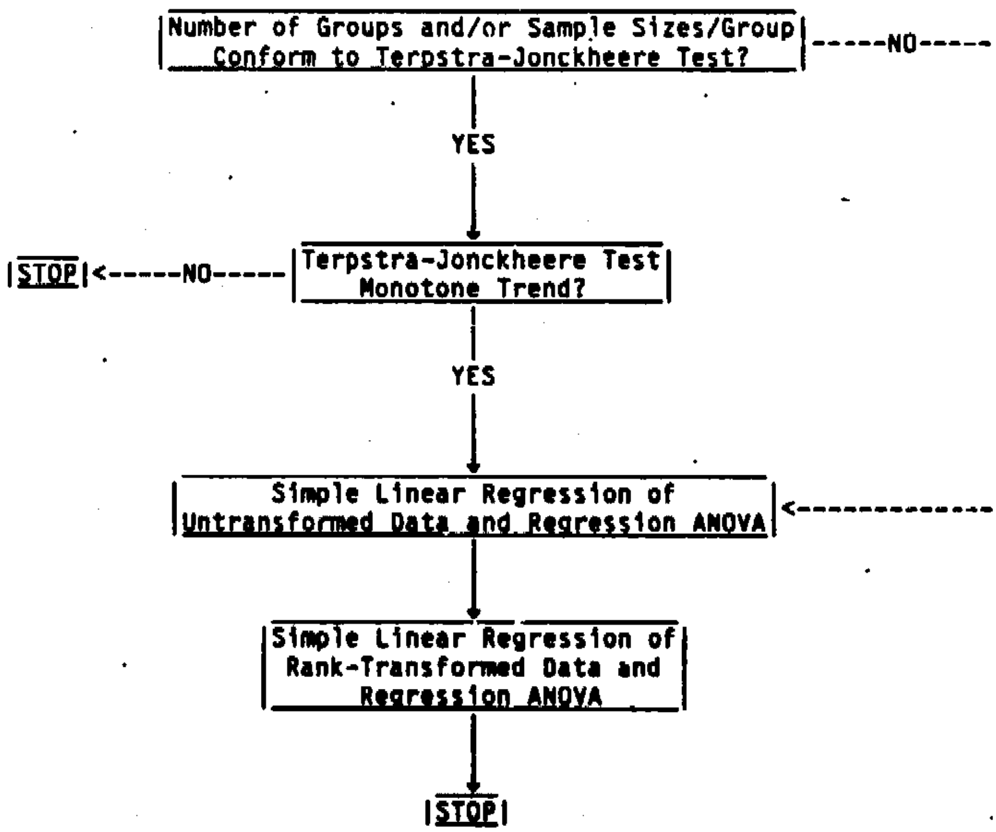


Figure 1C

LEVENE'S TEST OF HOMOGENEITY OF VARIANCES

↓ Homogeneous → ANOVA

↓ Heterogeneous

LOG₁₀ TRANSFORMATION ------Homogeneous------> ANOVA

↓ Heterogeneous

SQUARE TRANSFORMATION ------Homogeneous------> ANOVA

↓ Heterogeneous

SQUARE ROOT TRANSFORMATION ------Homogeneous------> ANOVA

↓ Heterogeneous

RECIPROCAL TRANSFORMATION ------Homogeneous------> ANOVA

↓ Heterogeneous

ANGULAR (ARCSINE) TRANSFORMATION ------Homogeneous------> ANOVA

↓ Heterogeneous

RANK TRANSFORMATION ------Homogeneous------> ANOVA

------Heterogeneous------> ANOVA

ANOVA

↓ Not Significant

TERPSTRA-JONCKHEERE NONPARAMETRIC TEST FOR TREND

↓ Significant

TERPSTRA-JONCKHEERE NONPARAMETRIC TEST FOR TREND

↓ Not Significant

DUNNETT'S CONTROL VS TREATMENT COMPARISONS (FOR EQUAL VARIANCES, FOR UNEQUAL VARIANCES, IF HETEROGENEOUS) 2-TAILED p = .05

↓ Significant

DUNNETT'S CONTROL VS TREATMENT COMPARISONS (FOR EQUAL VARIANCES, FOR UNEQUAL VARIANCES, IF HETEROGENEOUS) 1-TAILED p = .05

appendix pg 7

"Contains or relates to proprietary information of the Industry Task Force on 2,4-D Research Data"

HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE VIENNA VIRGINIA 22180 U.S.A.

005794

2184-101

either homogeneity was achieved or the entire series of transformations was used. When heterogeneity could not be removed, ANOVA of the rank-transformed data was completed.

When ANOVA (transformed or untransformed data) was significant, Dunnett's T-test was used to compare each group with the control. When ANOVA was not significant, no further analyses were conducted. Additionally, the Terpstra-Jonckheere nonparametric test for trend was performed on both homogeneous and heterogeneous data for the clinical hematology parameters and the organ weight data (see Figure 1C). All other data which were statistically evaluated for a possible trend (body weight values and food consumption) was analyzed according to the diagram presented in Figure 1B. Trend was evaluated by the Terpstra-Jonckheere test (monotone response) and by simple linear regression (linear response) of both untransformed and rank-transformed data.

Tests for homogeneity of variances and ANOVA were evaluated at the 5.0% one-tailed probability level. Control vs. compound-treated groups were evaluated at the 5.0% two-tailed probability level. If a significant trend was found, the groups were evaluated at the 5.0% one-tailed probability level. Data transformations and statistically significant differences and trends are designated throughout the summary tables by appropriate footnotes.

References are presented at the end of the text.

Specimen, Raw Data, and Final Report Storage

Specimens, raw data, and the final report are stored in the archives of Hazleton Laboratories America, Inc.

005794

RESULTS

Analytical Chemistry

Analytical chemistry data for each week of analyses are presented in Table 1.

Routine analyses conducted during the study revealed the following about 2,4-D concentrations.

Group	Sex	Percent of Target Range Low	Percent of Target Range High	Percent of Target Mean ± S.D.
2	Male	73.99	136.1	96.48 ± 14.824
2	Female	77.92	121.2	97.57 ± 10.677
3	Male	85.17	111.5	97.56 ± 6.490
3	Female	80.91	110.0	95.43 ± 8.234
4	Male	80.24	103.4	95.61 ± 4.966
4	Female	78.79	101.9	94.26 ± 5.347

Mortality and Clinical Observations

Adjusted survival data are summarized in Table 3 and graphically represented in Figure 2. An individual animal disposition list is presented in Appendix 5. Individual clinical signs are listed in Appendix 1 and summarized in Table 2.

Ten Group 1, six Group 2, 10 Group 3, and 11 Group 4 males were found dead or sacrificed *in extremis* during Weeks 1-104. One Group 4 male was found out of its cage at Week 30 and was removed from the study. Twelve Group 1, eight Group 2, 17 Group 3, and 15 Group 4 females were found dead or sacrificed *in extremis* during Weeks 1-104. One Group 2 female was found dead during Week 105 prior to its scheduled sacrifice. Three females (two Group 2 and one Group 3) were discovered to be missexed at Week 2 and were removed from the study. One Group 2 and one Group 3 female died due to accidental causes.

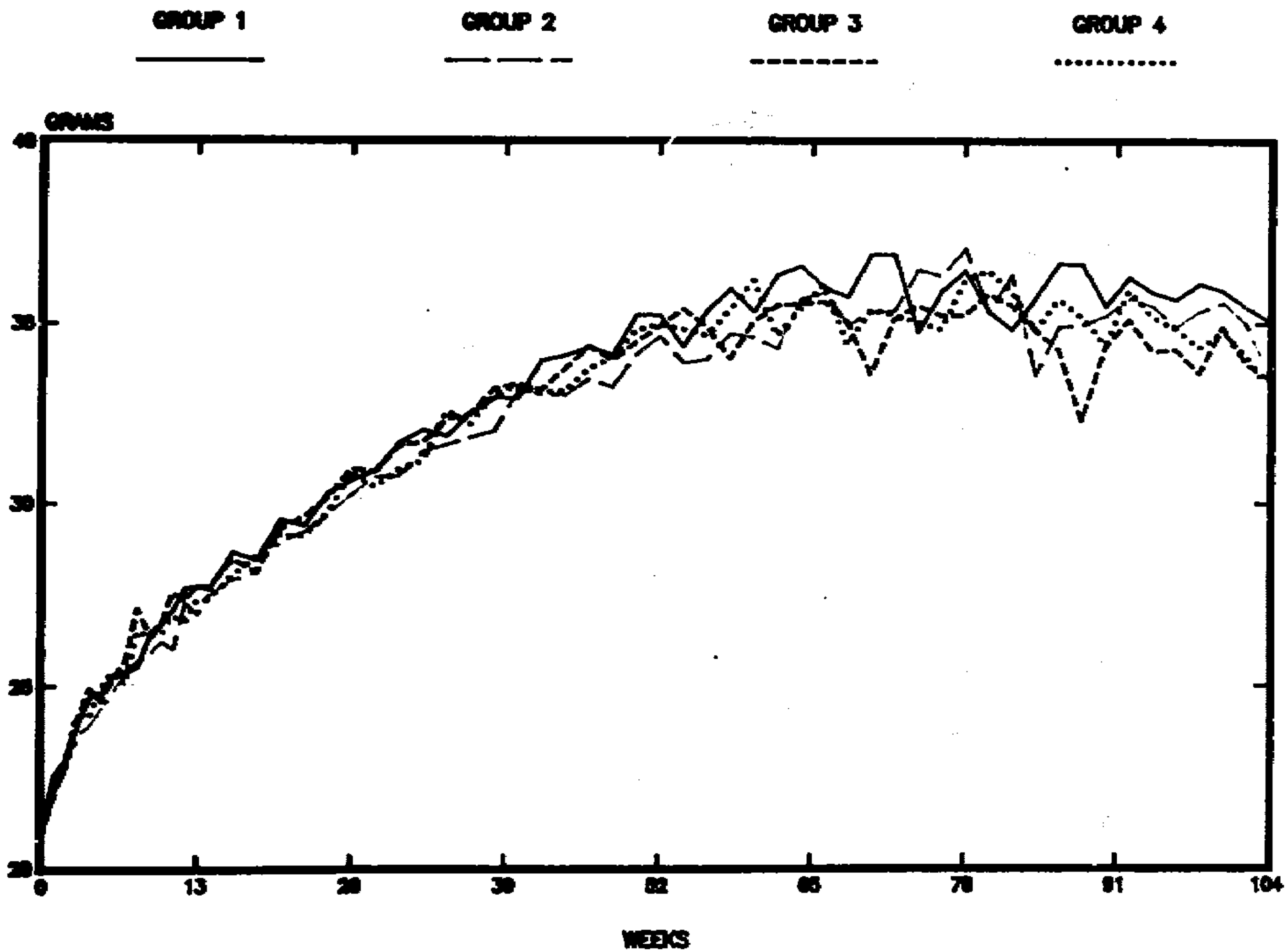
Analysis of survival revealed no statistically significant differences between the treated and control groups of either sex.

"Contains or relates to proprietary information of the Industry Task Force on 2,4-D Research Data"

HAZLETON LABORATORIES AMERICA, INC.

FIGURE 3 – MEAN BODY WEIGHTS

MALES 2184–101



- 26 -

0057794

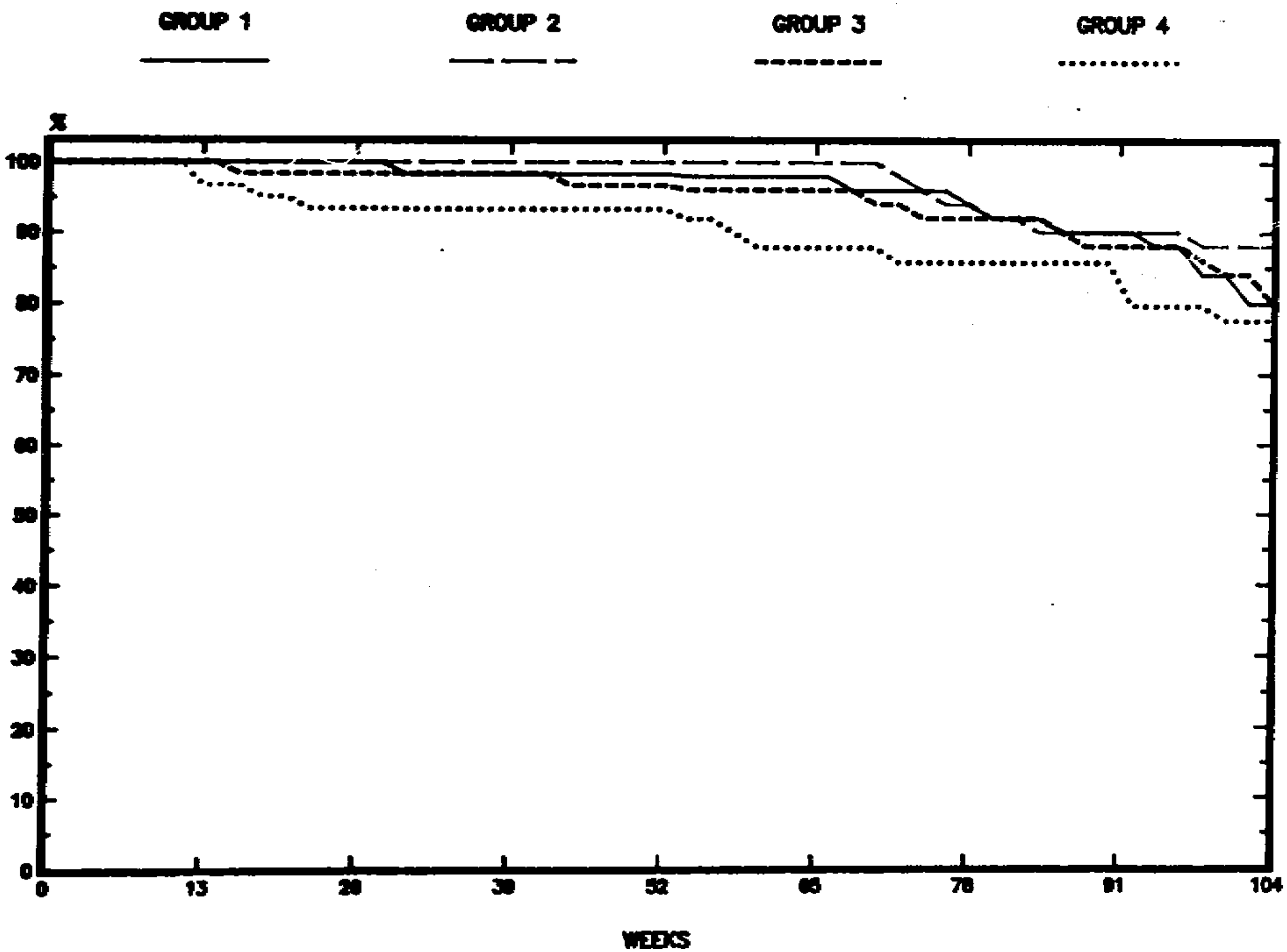
21

"Contains or relates to proprietary information of the Industry Task Force on 2,4-D Research Data"

HAZLETON

FIGURE 2 – ADJUSTED SURVIVAL

MALES 2184–101



- 23 -

005794

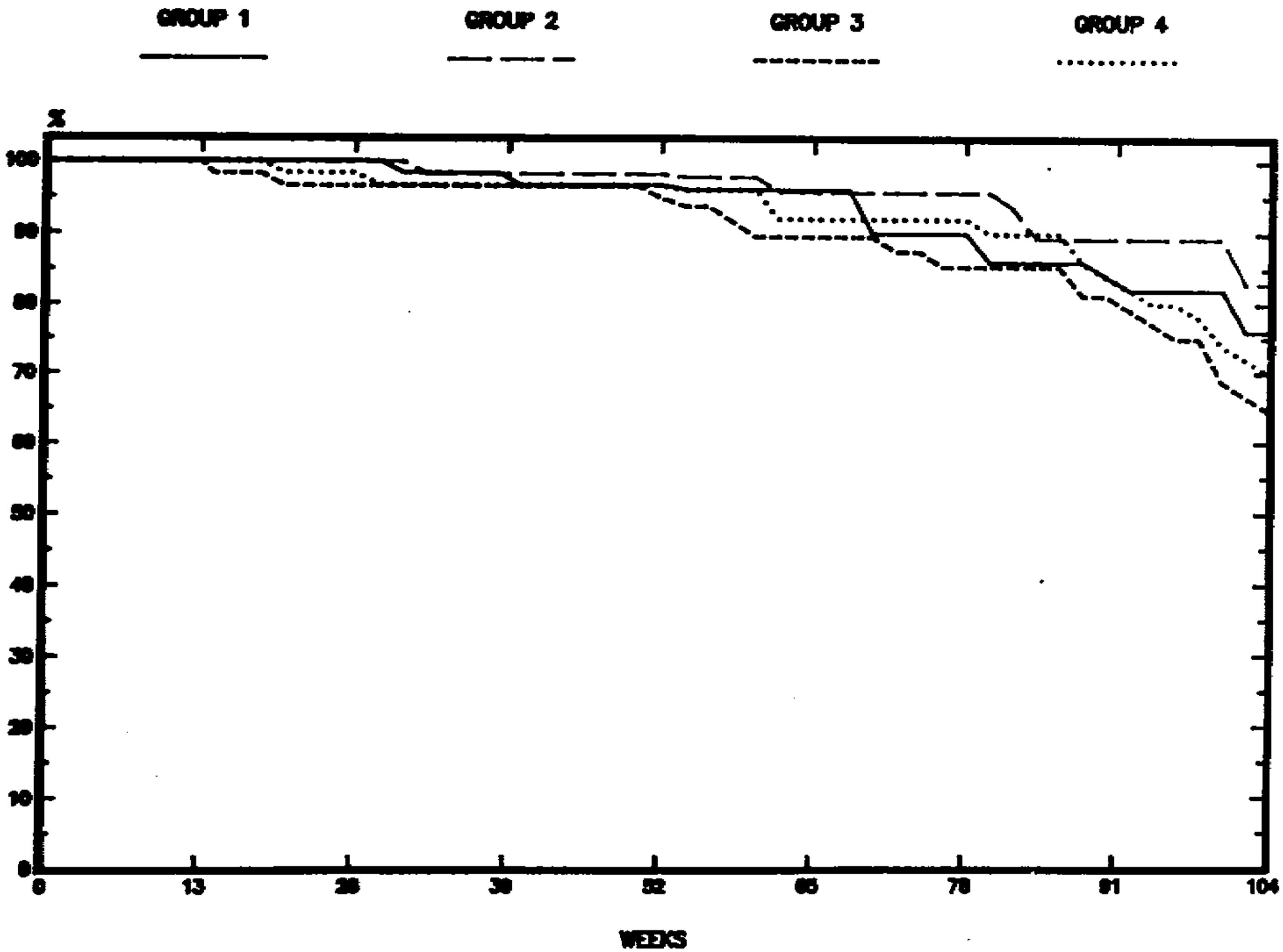
Appendix/88

HAZLETON LABORATORIES AMERICA, INC.

"Contains or relates to proprietary information of the Industry Task Force on 2,4-D Research Data"

FIGURE 2 – ADJUSTED SURVIVAL

FEMALES 2184–101



005794

Appendix pg 9

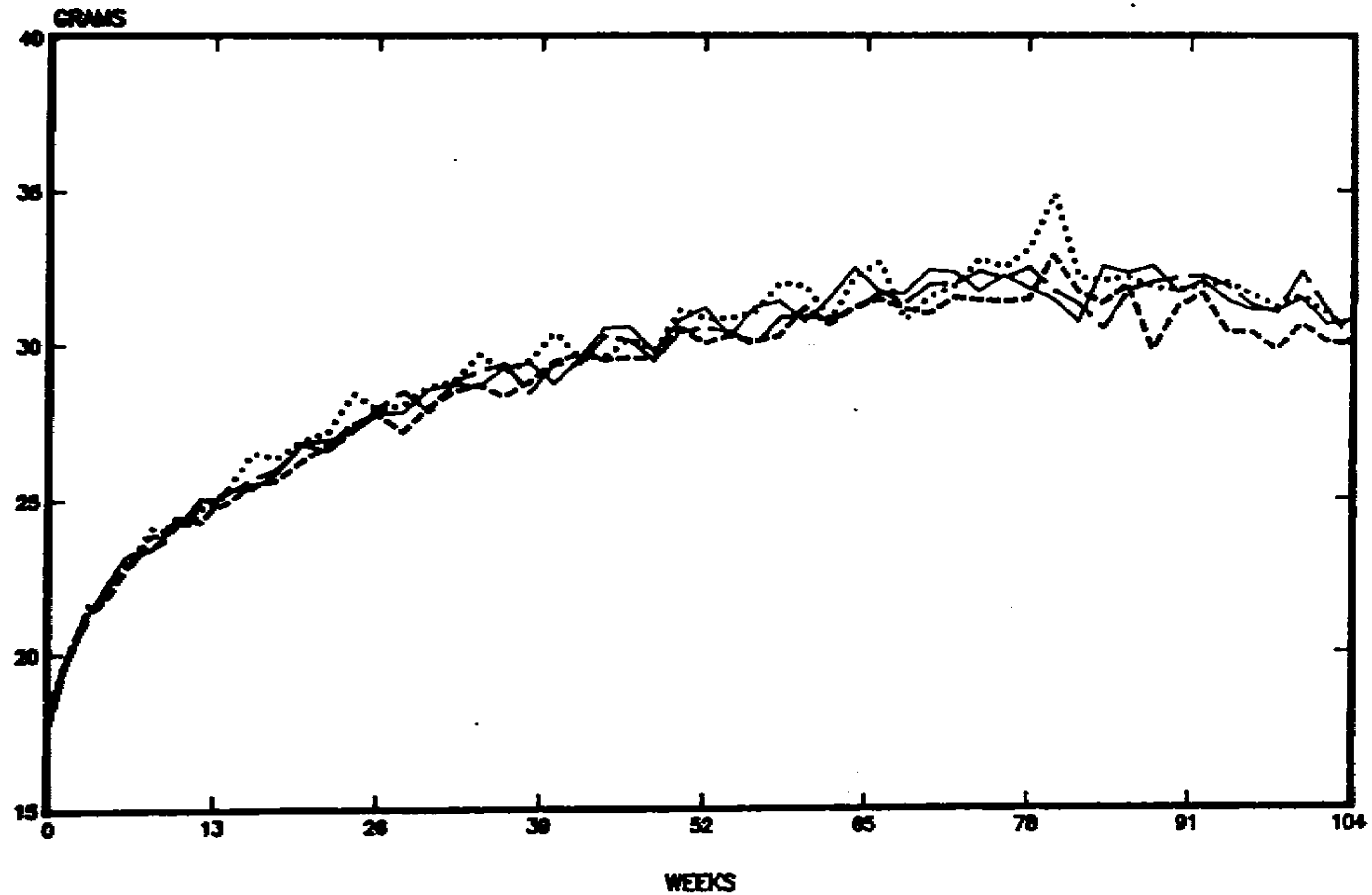
23

"Contains or relates to proprietary information of the Industry Task Force on 2,4-D Research Data"

FIGURE 3 – MEAN BODY WEIGHTS

FEMALES 2184–101

GROUP 1	GROUP 2	GROUP 3	GROUP 4
————	— — –	- - - - -	....



0057949

Appendix pg 11

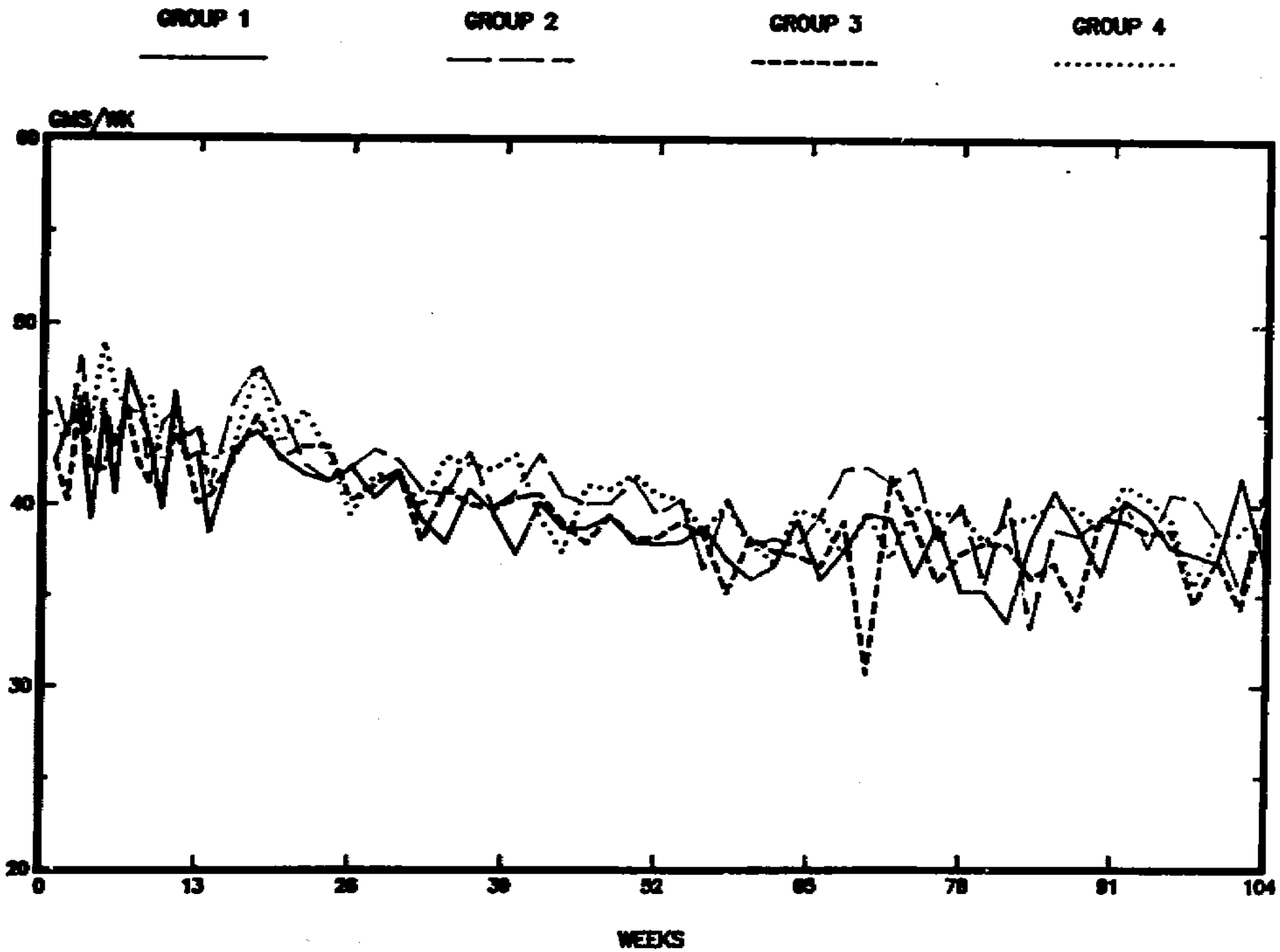
21

"Contains or relates to proprietary information of the Industry Task Force on 2,4-D Research Data"

HAZLETON LABORATORIES AMERICA INC.

FIGURE 4 — MEAN FOOD CONSUMPTION

MALES 2184-101



005794

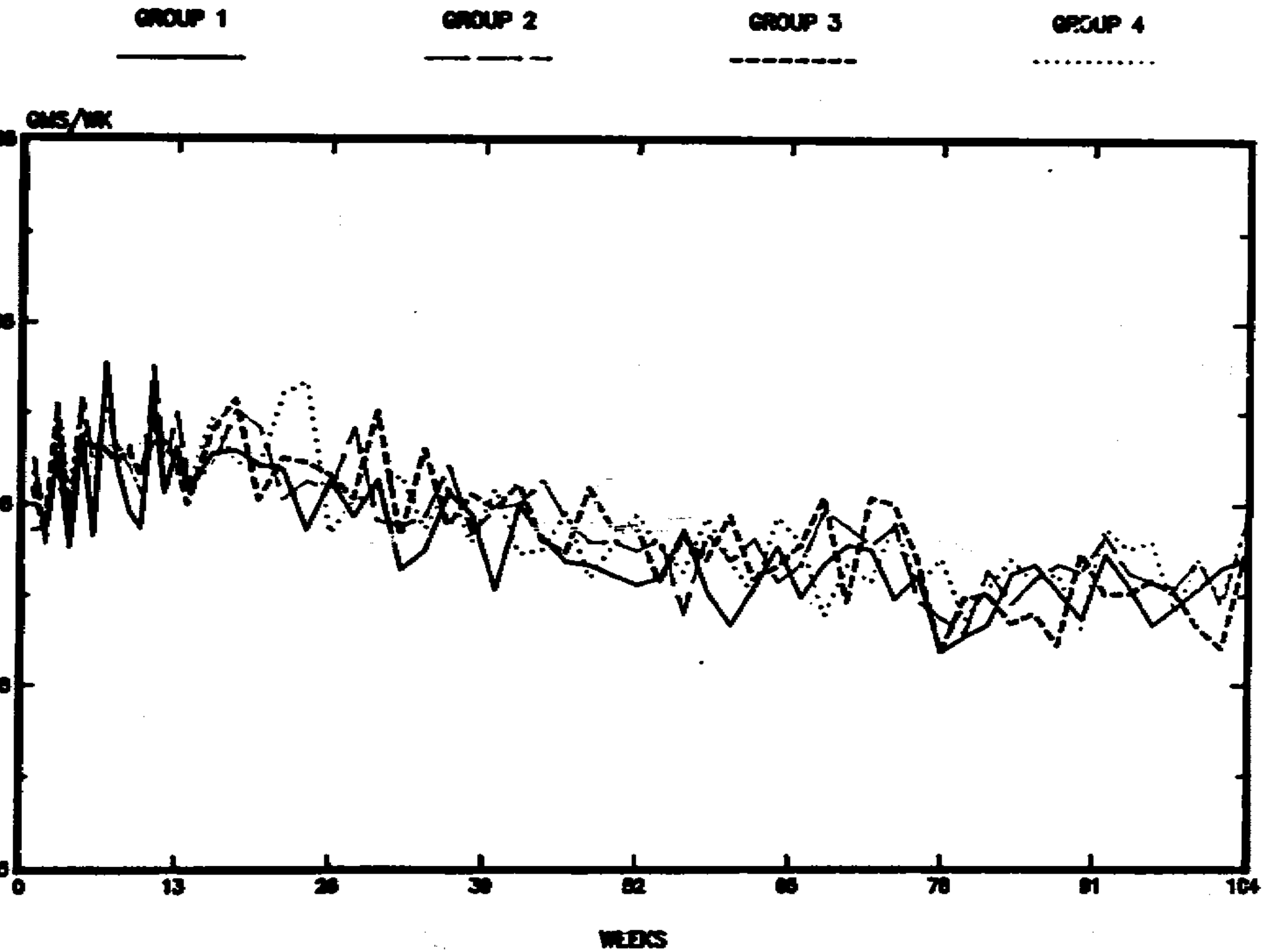
Appendix pg 12

"Contains or relates to propri...
information of the Industry Task
Force on 2,4-D Research Data"

HAZLETON

FIGURE 4 — MEAN FOOD CONSUMPTION

FEMALES 2184—101



- 29 -

005794

Appendix pg 13

26

END